

An amino acid sequence of human, type I, IMPDH protein

IMPDH = INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE 1
, 514 residues

MADYLISGGT GYVPEDGLTA QQLFASADDL TYNDFLILPG FIDFIADEVD LTSALTRKIT LKTPLISSPM DTVTEADMAI AMALMGGIGF IHHNCTPEFQ ANEVRKVKNF EQGFITDPVV LSPSHTVGDV LEAKMRHGFS GIPITETGTM GSKLVGIVTS RDIDFLAEKD HTTLLSEVMT PRIELVVAPA GVTLKEANEI LQRSKKGKLP IVNDCDELVA IIARTDLKKN RDYPLASKDS QKQLLCGAAV GTREDDKYRL DLLTQAGVDV IVLDSSQGNS VYQIAMVHYI KQKYPHLQVI GGNVVTAAQA KNLIDAGVDG LRVGMGCGSI CITQEVMACG RPQGTAVYKV AEYARRFGVP IIADGGIQTV GHVVKALALG ASTVMMGSLL AATTEAPGEY FFSDGVRLKK YRGMGSLDAM EKSSSSQKRY FSEGDKVKIA QGVSGSIQDK GSIQKFVPYL IAGIQHGCQD IGARSLSVLR SMMYSGELKF EKRTMSAQIE GGVHGLHSYE KRLY

An amino acid sequence of human, type II IMPDH protein

IMPDH = INOSINE-5'-MONOPHOSPHATE DEHYDROGENASE 2
, 514 residues

The underlined region correlates with the subdomain region

MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD LTSALTKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ ANEVRKVKKY EQGFITDPVV LSPKDRVRDV FEAKARHGFC GIPITDTGRM GSRLVGIISS RDIDFLKEEE HDCFLEEIMT KREDLVVAPA GITLKEANEI LQRSKKGKLP IVNEDDELVA IIARTDLKKN RDYPLASKDA KKQLLCGAAI GTHEDDKYRL DLLAQAGVDV VVLDSSQGNS IFQINMIKYI KDKYPNLQVI GGNVVTAAQA KNLIDAGVDA LRVGMGSGSI CITQEVLACG RPQATAVYKV SEYARRFGVP VIADGGIQNV GHIAKALALG ASTVMMGSLL AATTEAPGEY FFSDGIRLKK YRGMGSLDAM DKHLSSQNRY FSEADKIKVA QGVSGAVQDK GSIHKFVPYL IAGIQHSCQD IGAKSLTQVR AMMYSGELKF EKRTSSAQVE GGVHSLHSYE KRLF

The Subdomain of Wild-Type, Human, and IMPDH is Replaced with an Oligo-Peptide

3 Phe-514		
10 Leu-243	oligo	peptide
1et-1 Tyr-110		
~ [



The amino acid sequence of the modified IMPDH-DKT polypeptide 384 residues.

The substitute tri-peptide DKT sequence is highlighted in bold print

MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD
LTSALŢKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ
ANEVRKVKKY **DKT**LLCGAAI GTHEDDKYRL DLLAQAGVDV VVLDSSQGNS
IFQINMIKYI KDKYPNLQVI GGNVVTAAQA KNLIDAGVDA LRVGMGSGSI
CITQEVLACG RPQATAVYKV SEYARRFGVP VIADGGIQNV GHIAKALALG
ASTVMMGSLL AATTEAPGEY FFSDGIRLKK YRGMGSLDAM DKHLSSQNRY
FSEADKIKVA QGVSGAVQDK GSIHKFVPYL IAGIQHSCQD IGAKSLTQVR
AMMYSGELKF EKRTSSAQVE GGVHSLHSYE KRLF





The nucleotide sequence of type II, IMPDH-DKT cDNA

 $\verb|atggccgactacctgattagtggggcacgtcctacgtgccagacggactcacagcacagcagctct|$ teaactgeggagacggecteacctacaatgactttctcattctccctgggtacatcgacttcactgeaga $\verb|ccaggtggacctgacttctgaccaagaaaatcactcttaagaccccactggtttcctctcccatg|$ gacacagtcacagaggctgggatggccatagcaatggcgcttacaggcggtattggcttcatccaccaca actgtacacctgaattccaggccaatgaagttcggaaagtgaagaaatatgacaagaccctgctgtgtgg ggcagccattggcactcatgaggatgacaagtataggctggacttgctcgcccaggctggtgtggatgta gtggttttggactcttcccagggaaattccatcttccagatcaatatgatcaagtacatcaaagacaaat accctaatctcoaagtcattggaggcaatgtggtcactgctgcccaggccaagaacctcattgatgcagg tgtggatgccctgcgggtgggcatgggaagtggctccatctgcattacgcaggaagtgctggcctgtggg cggccccaagcaacagcagtgtacaaggtgtcagagtatgcacggcgctttggtgttccggtcattgctg atggaggaatccaaaatgtgggtcatattgcgaaagccttggcccttggggcctccacagtcatgatggg ctetetectggetgccaccactgaggcccctggtgaatacttettttccgatgggatccggetaaagaaa tatcgcggtatgggttctctcgatgccatggacaagcacctcagcagccagaacagatatttcagtgaag ctgacaaaatcaaagtggcccagggagtgtctggtgctgtgcaggacaaagggtcaatccacaaatttgt cccttacctgattgctggcatccaacactcatgccaggacattggtgccaagagcttgacccaagtccga gccatgatgtactctggggagcttaagtttgagaagagaacgtcctcagcccaggtggaaggtggcgtcc atagcctccattcgtatgagaagcggcttttctga



The amino acid sequence of the modified IMPDH-SPS polypeptide 384 residues.

The substitute tri-peptide SPT sequence is highlighted in bold print.

MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD
LTSALTKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ
ANEVRKVKKY SPSLLCGAAI GTHEDDKYRL DLLAQAGVDV VVLDSSQGNS
IFQINMIKYI KDKYPNLQVI GGNVVTAAQA KNLIDAGVDA LRVGMGSGSI
CITQEVLACG RPQATAVYKV SEYARRFGVP VIADGGIQNV GHIAKALALG
ASTVMMGSLL AATTEAPGEY FFSDGIRLKK YRGMGSLDAM DKHLSSQNRY
FSEADKIKVA QGVSGAVQDK GSIHKFVPYL IAGIQHSCQD IGAKSLTQVR
AMMYSGELKF EKRTSSAQVE GGVHSLHSYE KRLF



The nucleotide sequence of type II, IMPDH-SPS cDNA

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The amino acid sequence of the type II, modified $\ensuremath{\mathsf{IMPDH\text{-}GSG}}$ polypeptide

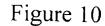
The substitute tri-peptide GSG sequence is highlighted in bold print

MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD LTSALTKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ ANEVRKVKKY **GSG**LLCGAAI GTHEDDKYRL DLLAQAGVDV VVLDSSQGNS IFQINMIKYI KDKYPNLQVI GGNVVTAAQA KNLIDAGVDA LRVGMGSGSI CITQEVLACG RPQATAVYKV SEYARRFGVP VIADGGIQNV GHIAKALALG ASTVMMGSLL AATTEAPGEY FFSDGIRLKK YRGMGSLDAM DKHLSSQNRY FSEADKIKVA QGVSGAVQDK GSIHKFVPYL IAGIQHSCQD IGAKSLTQVR AMMYSGELKF EKRTSSAQVE GGVHSLHSYE KRLF



The nucleotide sequence of type II, IMPDH-GSG cDNA

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The amino acid sequence of the modified IMPDH-SPT polypeptide 384 residues.

The substitute tri-peptide SPT sequence is highlighted in bold print.

MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD LTSALTKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ ANEVRKVKKY SPTLLCGAAI GTHEDDKYRL DLLAQAGVDV VVLDSSQGNS IFQINMIKYI KDKYPNLQVI GGNVVTAAQA KNLIDAGVDA LRVGMGSGSI CITQEVLACG RPQATAVYKV SEYARRFGVP VIADGGIQNV GHIAKALALG ASTVMMGSLL AATTEAPGEY FFSDGIRLKK YRGMGSLDAM DKHLSSQNRY FSEADKIKVA QGVSGAVQDK GSIHKFVPYL IAGIQHSCQD IGAKSLTQVR AMMYSGELKF EKRTSSAQVE GGVHSLHSYE KRLF



The nucleotide sequence of type II, IMPDH-SPT cDNA

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The nucleotide sequence of type II, IMPDH-SPTQ cDNA

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The amino acid sequence of the modified type II, IMPDH-AGRP polypeptide 385 residues

The substitute tetra-peptide AGRP sequence is highlighted in bold print.

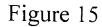
MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD
LTSALTKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ
ANEVRKVKKY AGRPLLCGAA IGTHEDDKYR LDLLAQAGVD VVVLDSSQGN
SIFQINMIKY IKDKYPNLQV IGGNVVTAAQ AKNLIDAGVD ALRVGMGSGS
ICITQEVLAC GRPQATAVYK VSEYARRFGV PVIADGGIQN VGHIAKALAL
GASTVMMGSL LAATTEAPGE YFFSDGIRLK KYRGMGSLDA MDKHLSSQNR
YFSEADKIKV AQGVSGAVQD KGSIHKFVPY LIAGIQHSCQ DIGAKSLTQV
RAMMYSGELK FEKRTSSAQV EGGVHSLHSY EKRLF





The nucleotide sequence of type II, IMPDH-AGRP

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The amino acid sequence of type II, modified ${\tt IMPDH-NSPL}$ polypeptide

The substitute tri-peptide is highlighted in bold print

MADYLISGGT SYVPDDGLTA QQLFNCGDGL TYNDFLILPG YIDFTADQVD
LTSALTKKIT LKTPLVSSPM DTVTEAGMAI AMALTGGIGF IHHNCTPEFQ
ANEVRKVKKY NSPLLLCGAA IGTHEDDKYR LDLLAQAGVD VVVLDSSQGN
SIFQINMIKY IKDKYPNLQV IGGNVVTAAQ AKNLIDAGVD ALRVGMGSGS
ICITQEVLAC GRPQATAVYK VSEYARRFGV PVIADGGIQN VGHIAKALAL
GASTVMMGSL LAATTEAPGE YFFSDGIRLK KYRGMGSLDA MDKHLSSQNR
YFSEADKIKV AQGVSGAVQD KGSIHKFVPY LIAGIQHSCQ DIGAKSLTQV
RAMMYSGELK FEKRTSSAQV EGGVHSLHSY EKRLF



The nucleotide sequence of type II, IMPDH-NSPL cDNA

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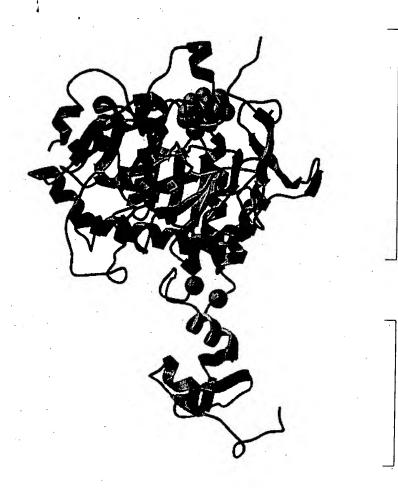


The amino acid sequence of the type I modified IMPDH-DKT polypeptide

The substitute tri-peptide DKT is highlighted in bold print

MADYLISGGT GYVPEDGLTA QQLFASADGL TYNDFLILPG FIDFIADEVD LTSALTRKIT LKTPLISSPM DTVTEADMAI AMALMGGIGF IHHNCTPEFQ ANEVRKVKKF DKTLLCGAAV GTREDDKYRL DLLTQAGVDV IVLDSSQGNS VYQIAMVHYI KQKYPHLQVI GGNVVTAAQA KNLIDAGVDG LRVGMGCGSI CITQEVMACG RPQGTAVYKV AEYARRFGVP IIADGGIQTV GHVVKALALG ASTVMMGSLL AATTEAPGEY FFSDGVRLKK YRGMGSLDAM EKSSSQKRY FSEGDKVKIA QGVSGSIQDK GSIQKFVPYL IAGIQHGCQD IGARSLSVLR SMMYSGELKF EKRTMSAQIE GGVHGLHSYE KRLY

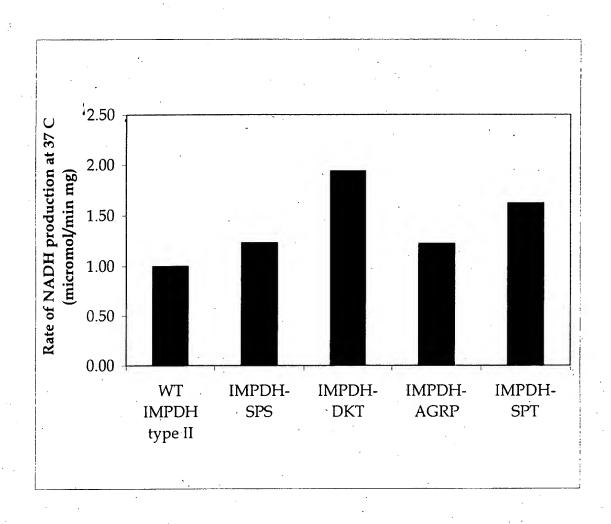
A schematic representation of the distance that the substitute oligo-peptides are designed to span in a folded modified IMPDH polypeptide.



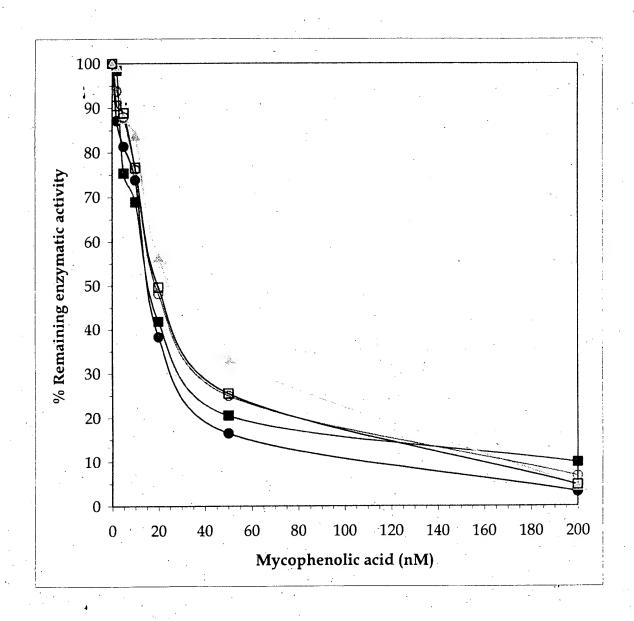
CATALYTIC CORE DOMAIN

SUBDOMAIN

The Rate of NADH Production at 37 $^{\circ}\text{C}$ for Wild-Type IMPDH (type II) and Various Modified IMPDH Multimers.

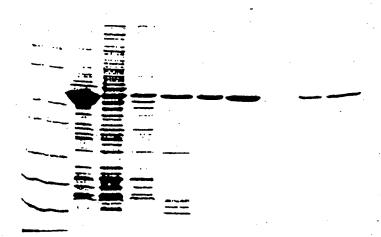


Various Activity of Modified IMPDH MPA Inhibits the Polypeptides.



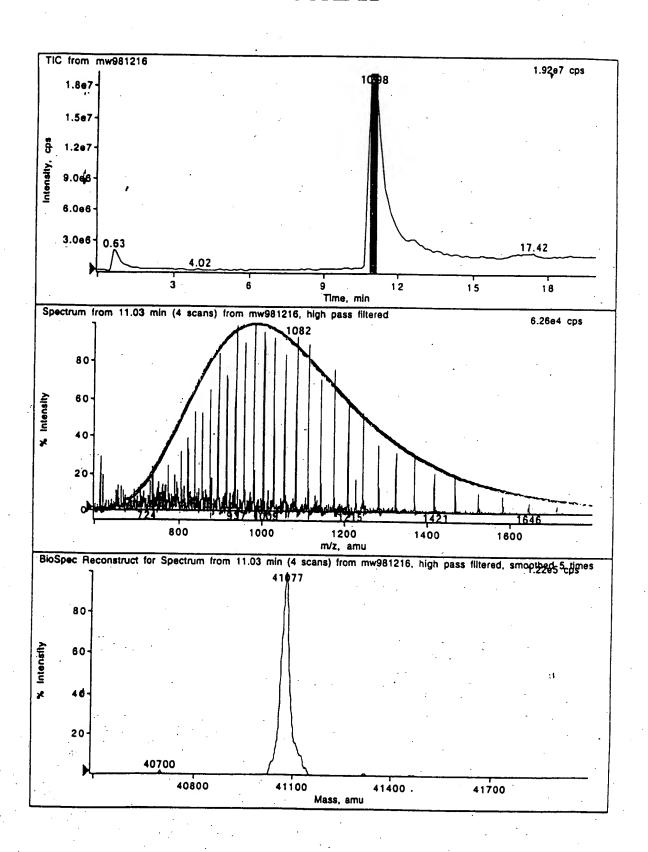
- wild-type, type II IMPDH type II, IMPDH-AGRP
- type II, IMPDH-SPS
- type II, IMPDH-SPT
- type II, IMPDH-DKT

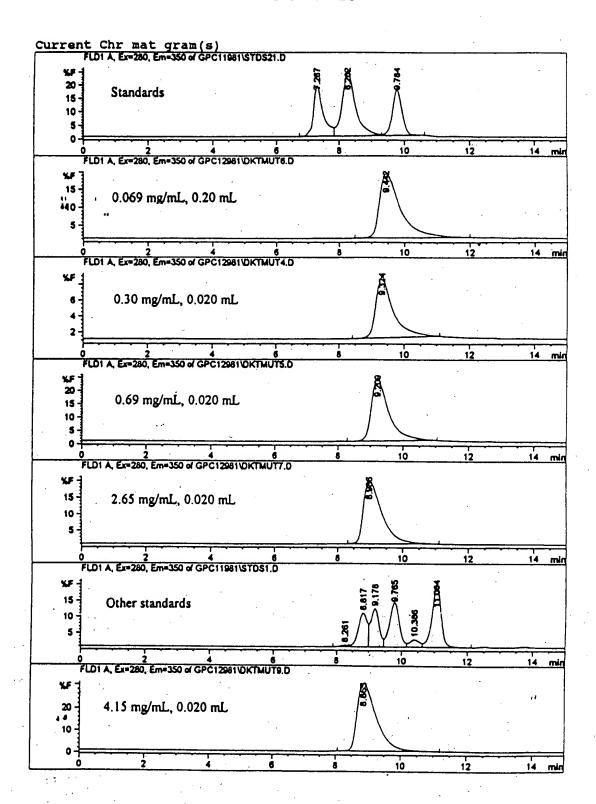
1 2 3 4 5 6 7 8 9 10



Lane #

- 1: Novex standards (25 µL)
- 2: Total cell lysate (1 μL, 12 μg; before ultracentrifugation)
- 3: Soluble lysate (2.5 μ L, 10 μ g; after 100,000 x g, 1 hr, 4 °C)
- 4: Unbound AE sample (13 μL, ~10 μg)
- 5: Blue dye column, frs. #32-70 (25 μL, 4.5 μg)
- 6: IMP affinity column, IMP eluted (10 μ L, ~1.5 μ g)
- 7: IMP affinity column, IMP eluted (20 µL, ~3.0 µg)
- 8: Unbound protein to IMP column (25 μ L, ~2.0 μ g)
- 9: IMP affinity column (from AE fr. #1-10, $10 \mu L$, $1.2 \mu g$)
- 10: IMP affinity column (from AE fr. #1-10, 25 μL, 3.0 μg)







The nucleotide sequence of type I, IMPDH-DKT

 $\verb|atggcggactacctgatcagcggcggcaccggctacgtgcccgaggatgggctcaccgcgcagcagctct|$ tcgccagcgccgacggcctcacctacaacgacttcctgattctcccaggattcatagacttcatagctga tgaggtggacctgacctgacctgacccggaagatcacgctgaagacgccactgatctcctccccatg actgcaccccagagttccaggccaacgaggtgcggaaggtcaagaagtttgacaaaaccctgctctgtgg atagtettggaetegteceaagggaatteggtgtateaaategeeatggtgeattacateaaacagaagt tgtggacgggctgcgcttgcgctccatctgcatcacccaggaagtgatggcctgtggt cggccccagggcactgctgtgtacaaggtggctgagtatgcccggcgctttggtgtgcccatcatagccg atggcggcatccagaccgtgggacacgtggtcaaggccctggcccttggagcctccacagtgatgatggg ctccctgctggccgccactacggaggcccctggcgagtacttcttctcagacggggtgcggctcaagaag taccggggcatgggctcactggatgccatggagaagagcagcagcagcagaaacgatacttcagcgagg gecetaceteatageaggeatecaacaggetgecaggatateggggecegeageetgtetgteettegg tccatgatgtactcaggagagctcaagtttgagaagcggaccatgtcggcccagattgagggtggtgtcc atggcctgcactcttacgaaaagcggctgtactga